

**Aging and Cardiovascular Function**

# Effects of Aging on Cardiovascular Responses to Parasympathetic Withdrawal

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| <b>OBJECTIVES</b>  | The study was done to determine whether the effects of parasympathetic withdrawal on heart rate, blood pressure (BP), and systolic and diastolic function are altered with normal aging.   |
| <b>BACKGROUND</b>  | Cardiac responses to beta-adrenergic sympathetic stimulation decline with aging as does the heart rate response to parasympathetic withdrawal, but the extent to which other responses to parasympathetic withdrawal decrease is less clear.   |
| <b>METHODS</b>     | Heart rate, BP, systolic function, and diastolic filling responses to parasympathetic withdrawal induced by atropine (0.02 mg/kg) were compared in 50 healthy subjects, 28 older (ages 65 to 80 years, mean 70 years; 18 females all on estrogen) and 22 young (age 18 to 32 years, mean 26 years; 12 females) subjects, using radionuclide angiography.   |
| <b>RESULTS</b>     | Parasympathetic withdrawal in the older group caused less of an increase in heart rate (+33 vs. +48 beats/min), cardiac index (+0.6 vs. +1.5 l/m <sup>2</sup> ), systolic blood pressure (−1 vs. +7 mm Hg), and early diastolic filling rate (+1.7 vs. +2.4 end-diastolic volumes/s) (all $p \leq 0.01$ ). At similar declines in the diastolic filling period, end-diastolic volume index (EDVI) fell substantially more in the older group (−11.6 vs. −2.4 ml/m <sup>2</sup> , $p < 0.001$ ). The only gender difference was in diastolic filling rate, which was similar in the young males and females, but significantly less in older males than in older females. |
| <b>CONCLUSIONS</b> | The responses to parasympathetic withdrawal as well as sympathetic stimulation decline with aging, and both contribute to the reduced cardiovascular responses to stress with advancing age. (J Am Coll Cardiol 2003;41:2077–83) © 2003 by the American College of Cardiology Foundation   |

The marked increase in cardiac output during exercise or other stress depends on increases in heart rate and stroke volume. Increased beta-adrenergic stimulation and withdrawal of parasympathetic tone largely govern these changes. With aging, in the absence of disease, sympathetic control of the heart declines; this is manifested by reduced heart rate, blood pressure (BP), ejection fraction (EF), and cardiac output responses to beta-adrenergic stimulation (1,2). Although the heart rate response is known to decline with aging (3,4), little is known about the impact of aging on other cardiovascular responses to parasympathetic withdrawal.

Parasympathetic control of cardiovascular function includes marked effects on heart rate and the conduction system and lesser effects on atrial and ventricular contractility. Vagal activity inhibits the sinoatrial node, atrial myocardium, and atrioventricular conduction tissue and also depresses ventricular myocardium (5–9). There is conflicting evidence about whether parasympathetic control of

cardiac function changes with age. In rat models, evidence with aging shows both an increased and reduced sensitivity to vagal activity (10–12). In humans, evidence of an age-related decrease in parasympathetic modulation includes studies of heart rate variability (HRV) and baroreflex sensitivity, which decline with aging (3,13–19). There is a reduced heart rate response to parasympathetic withdrawal with aging (3,4), but whether contractility or diastolic filling responses are altered is unknown. A recent study suggested important age-related differences in BP control due to age differences in vagal inhibition of heart rate and thereby cardiac output (20). In addition, whether gender influences parasympathetic control of cardiovascular function is unknown.

In the current study, vagal blockade induced by atropine was used to investigate age differences in cardiac parasympathetic regulation. The purpose of this study was to determine the influence of aging on contractile, BP, and diastolic filling responses to parasympathetic withdrawal. We hypothesized that aging would be associated with lesser changes in systolic and diastolic measures with parasympathetic withdrawal due to a lower baseline vagal tone. We also sought to determine whether gender differences exist in the effects of parasympathetic withdrawal. Our results document that normal aging in the absence of disease is associated with lesser parasympathetic regulation of heart rate, cardiac output, and diastolic filling.

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#### Abbreviations and Acronyms

|       |   |
|-------|---|
| BP    | = blood pressure  |
| CI    | = cardiac index   |
| EDV   | = end-diastolic volume                                      |
| EDVI  | = end-diastolic volume index                                |
| EF    | = ejection fraction   |
| ESVI  | = end-systolic volume index                                 |
| HRV   | = heart rate variability                                    |
| PEFR  | = peak early filling rate                                   |
| RMSSD | = root-mean square of difference of successive RR intervals |
| SVI   | = stroke volume index                                       |

## METHODS

**Subject selection.** Healthy males and females age 18 to 33 or 65 to 80 years who were sedentary (no regular exercise >20 min >2 times weekly) were screened. Exclusion criteria included current smoking, hypertension, any cardiovascular disease, exercise-limiting arthritis or pulmonary disease, obesity (body mass index  $\geq 30$ ), and chronic use of any prescription medication except for treated hypothyroidism ( $n = 2$ ) and estrogen replacement therapy, which all older females received. Inclusion criteria were an unremarkable medical history, physical examination, blood tests (complete blood count, sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, albumin, serum glutamic-oxaloacetic transaminase, alkaline phosphatase, lactic dehydrogenase, cholesterol, thyroid stimulating hormone), urinalysis, urine pregnancy test (young females), normal echocardiogram (no more than mild regurgitation), and normal Bruce protocol maximal exercise testing, which included normal postexercise single-photon emission computed tomography technetium-99m sestamibi imaging in all older subjects. Twenty-eight older (mean = 70 years, range 65 to 80 years, 18 females and 10 males) and 22 younger (mean age = 26 years, range 18 to 32 years, 13 females and 9 males) healthy volunteers were studied. The study was approved by the Human Subjects Committee of the University of Washington. Procedures followed were in accordance with institutional guidelines. All subjects gave informed written consent.

**Study protocol.** Following placement of intravenous catheters, subjects rested supine for 30 min. Two bolus intravenous injections of atropine were administered for a total of 0.02 mg/kg (0.01 mg/kg initially and repeated in 6 min). Cardiac blood pool images, heart rate, and BP were recorded at rest and beginning 3 min after each atropine injection.

**Data collection and processing. RADIONUCLIDE ANGIOGRAPHY.** Red blood cells were labeled with Tc-99m and images acquired in the left anterior oblique projection, which offered the best septal definition with a high sensitivity parallel hole collimator, 32 frames, and a  $\pm 20\%$  arrhythmia rejection. The resting acquisition was for 15 million counts and subsequent acquisitions were for 3 min.

The EF and cardiac volumes were calculated using modifications of previously described and validated methods (21). Image data were excluded in two subjects (both young females) because of poor image quality due to a poor red blood cell tag. Volumes were divided by the body surface area to derive end-diastolic volume index (EDVI), end-systolic volume index (ESVI), and stroke volume index (SVI). Cardiac index (CI) was obtained by multiplying the SVI times the mean heart rate during the acquisition. The peak early filling rate (PEFR) was measured as previously described (22) and expressed in both ml/s/m<sup>2</sup> and in EDV/s. The diastolic filling time was calculated by subtracting the systolic emptying period in ms from the RR interval.

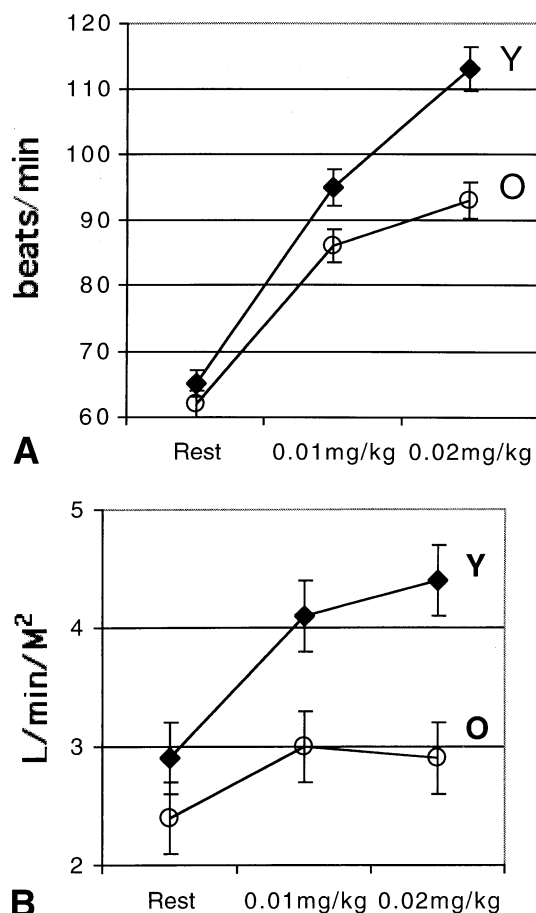
**HRV.** Two-channel Holter recordings were obtained in 10 older and 14 younger subjects to document that the atropine doses used caused parasympathetic withdrawal as measured by HRV. Tapes were analyzed with a Spacelabs Model FT2000 (Issaquah, Washington). Two minutes of recording were performed at rest and during the radionuclide angiograms. The root-mean square of difference of successive RR intervals (RMSSD) was used as the measure of HRV.

**Statistical analysis.** Results are expressed as the mean  $\pm$  SE. The effects of age and gender were compared using analysis of variance for repeated measures, which included rest data and data after each atropine dose (StatView 5.0, Abacus, Berkeley, California). The reported *p* values are for the effects of atropine and the interaction term (old vs. young  $\times$  atropine or males vs. females  $\times$  atropine). An unpaired *t* test was used to compare the percentage change from rest to the final atropine dose between groups. Statistical significance was established at  $p \leq 0.05$ .

## RESULTS

**Effects of aging on heart rate and cardiac systolic function responses.** Heart rate increased significantly less in the old than in the young (52% vs. 74%,  $p < 0.0001$ ) (Fig. 1, Table 1). Parasympathetic withdrawal in these resting subjects caused no change in EF. The EDVI, ESVI, and SVI fell significantly in both groups, but there was no difference between age groups. Because of the lesser increase in heart rate in the older subjects and the somewhat greater decline in SVI, the CI increased less in the older group (22% vs. 53%,  $p < 0.0001$ ). The CI increased by 0.6 l/min/m<sup>2</sup> in the older group compared to 1.5 l/min/m<sup>2</sup> in the young. Systolic and mean BP increased less in the old than in the young (both  $p < 0.001$ ). The diastolic BP response was not different between the two groups.

**Effects of aging on diastolic filling and end-diastolic volume (EDV) responses.** Parasympathetic withdrawal increased diastolic filling rates significantly ( $p \leq 0.0001$ ). However, the increase in diastolic filling rates with parasympathetic withdrawal was attenuated with aging (Table 2). The mean PEFR in ml/s/m<sup>2</sup> increased by 64 in the old compared with 120 in the young, and the mean PEFR in



**Figure 1.** Heart rate (A) and cardiac index (B) ( $\pm$  SEM) in the young (Y) and older (O) groups at rest and following atropine ( $p < 0.0001$  for old vs. young  $\times$  atropine dose).

EDV/s increased 1.7 in the older group compared with 2.4 in the young (both  $p \leq 0.04$  age group  $\times$  dose).

The EDVI was more adversely affected by tachycardia in the older group (Fig. 2). To assess changes in diastolic volume at similar decrements in diastolic filling time, we compared the results in the older group after the second atropine dose, when the heart rate had increased to 94 beats/min, to the results in the younger group after the first atropine dose when subjects' heart rate had increased similarly to 96 beats/min. The diastolic filling periods were similar in the old and young at these heart rates ( $340 \pm 13$  ms old vs.  $349 \pm 15$  ms young,  $p = \text{ns}$ ). At these similar heart rates and diastolic filling periods, the fall in EDVI in the older group was nearly fivefold greater than in the young ( $-11.6 \pm 1.6$  ml/m<sup>2</sup> vs.  $-2.4 \pm 1.6$  ml/m<sup>2</sup>,  $p = 0.04$ ). Thus, tachycardia impaired ventricular filling more with aging as measured by peak early filling rates and by the decline in EDVI at similar decrements in the diastolic filling period.

**Gender effects.** No gender differences existed in heart rate, BP, EF, EDVI, ESVI, SVI, or CI responses to parasympathetic withdrawal. Although parasympathetic withdrawal increased diastolic filling rates in both genders, the increase

was less in males than in females as measured in either EDV/s or ml/s/m<sup>2</sup> (both  $p < 0.05$  for gender  $\times$  dose) (Table 2). This gender difference was largely due to a difference in the older men compared with older women, because the younger men and women had similar responses (Table 2, Fig. 3).

**HRV.** The HRV as measured by the RMSSD declined with atropine ( $p \leq 0.0001$ ). The response to atropine was less in the old ( $26 \pm 7$  at rest to  $11 \pm 3$  ms after the first dose to  $18 \pm 9$  ms after the second dose) than in the young ( $53 \pm 8$  to  $10 \pm 2$  to  $8 \pm 0.2$  ms) ( $p < 0.002$ ), owing to a substantially lower resting HRV in the older group. No gender difference existed in the HRV response.

## DISCUSSION

This study investigated differences in heart rate, systolic, and diastolic responses to parasympathetic withdrawal as a function of aging. Aging reduced the effects of parasympathetic withdrawal on heart rate, CI, systolic BP, mean BP, and diastolic filling. These results document that parasympathetic modulation of cardiovascular function is influenced by normal aging. Mechanisms underlying the reduced cardiac vagal control with aging could occur at several levels. In humans, M<sub>2</sub> muscarinic receptor density is reduced with aging, and there is decreased receptor function (4). Muscarinic receptor activity is probably reduced with aging (23), and impaired cardiac acetylcholine release occurs in response to stimulation (24). Whether human acetylcholinesterase levels change with aging is not clear.

**Aging differences. HEART RATE AND CI.** This study and prior studies document that the resting heart rate is under less parasympathetic control with advancing age (3,4). The likely major underlying factor in the reduced response to parasympathetic withdrawal with aging is the age-associated reduction in baseline parasympathetic activity. Parasympathetic modulation of heart rate as estimated by the baroreflex response (25) or HRV (13,16,26) decreases with aging. Fukusaki et al. (15) concluded that the age-related decline in HRV was primarily mediated by aging per se and not by physiologic changes of normal aging such as fitness, body habitus, obesity, or BP. In the current study, older subjects had reduced HRV at rest, and a lesser change in HRV with atropine. The reduced cardiac output response to parasympathetic withdrawal with aging is almost entirely due to the lesser increase in heart rate, as stroke volume responses do not change significantly with age.

**CONTRACTILITY AND BP.** Although vagal activity has been shown to inhibit contractile function at rest in animal models and in isolated human right atrial strips (27), vagal inhibition of ventricular contractility in humans has only been demonstrated under conditions of increased sympathetic stimulation (5,28,29). Earlier studies have documented a moderately increased sympathetic tone with aging (30). Despite this, we found no age difference in ventricular contractile responses to parasympathetic withdrawal with

**Table 1.** Mean Responses to Atropine (Mean  $\pm$  SE) in Young and Older Subjects

|                           | Rest          | 0.01 mg/kg    | 0.02 mg/kg    | ANOVA p Values |                                | Mean % Change<br>0.02 mg/kg vs. Rest |
|---------------------------|---------------|---------------|---------------|----------------|--------------------------------|--------------------------------------|
|                           |               |               |               | Dose<br>Effect | Young vs.<br>Old $\times$ Dose |                                      |
| Heart rate (beats/min)    |               |               |               |                |                                |                                      |
| Young                     | 65 $\pm$ 1    | 96 $\pm$ 2    | 113 $\pm$ 2   | $\leq 0.0001$  | $\leq 0.0001$                  | 74 $\pm$ 4                           |
| Older                     | 61 $\pm$ 1    | 86 $\pm$ 2    | 94 $\pm$ 2    |                |                                | 52 $\pm$ 4*                          |
| Systolic BP (mm Hg)       |               |               |               |                |                                |                                      |
| Young                     | 119 $\pm$ 2   | 124 $\pm$ 3   | 126 $\pm$ 3   | 0.008          | $\leq 0.001$                   | 6 $\pm$ 1                            |
| Older                     | 129 $\pm$ 3   | 129 $\pm$ 3   | 128 $\pm$ 2   |                |                                | -1 $\pm$ 1*                          |
| Diastolic BP (mm Hg)      |               |               |               |                |                                |                                      |
| Young                     | 73 $\pm$ 1    | 78 $\pm$ 1    | 77 $\pm$ 2    | $\leq 0.0001$  | NS                             | 9 $\pm$ 2                            |
| Older                     | 73 $\pm$ 1    | 78 $\pm$ 2    | 80 $\pm$ 2    |                |                                | 6 $\pm$ 2                            |
| Mean BP (mm Hg)           |               |               |               |                |                                |                                      |
| Young                     | 88 $\pm$ 1    | 93 $\pm$ 2    | 95 $\pm$ 2    | $\leq 0.0001$  | $\leq 0.02$                    | 8 $\pm$ 1                            |
| Older                     | 92 $\pm$ 2    | 95 $\pm$ 2    | 94 $\pm$ 2    |                |                                | 2 $\pm$ 2*                           |
| EF                        |               |               |               |                |                                |                                      |
| Young                     | 65 $\pm$ 2    | 65 $\pm$ 2    | 64 $\pm$ 2    | NS             | NS                             | -3 $\pm$ 1                           |
| Older                     | 61 $\pm$ 2    | 61 $\pm$ 2    | 60 $\pm$ 2    |                |                                | -1 $\pm$ 2                           |
| EDVI (ml/m <sup>2</sup> ) |               |               |               |                |                                |                                      |
| Young                     | 68 $\pm$ 4    | 66 $\pm$ 4    | 60 $\pm$ 4    | $\leq 0.0001$  | NS                             | -11 $\pm$ 4                          |
| Older                     | 65 $\pm$ 3    | 56 $\pm$ 4    | 53 $\pm$ 3    |                |                                | -15 $\pm$ 3                          |
| ESVI (ml/m <sup>2</sup> ) |               |               |               |                |                                |                                      |
| Young                     | 24 $\pm$ 2    | 23 $\pm$ 2    | 22 $\pm$ 2    | $\leq 0.0001$  | NS                             | -6 $\pm$ 5                           |
| Older                     | 25 $\pm$ 2    | 22 $\pm$ 2    | 21 $\pm$ 2    |                |                                | -16 $\pm$ 3                          |
| SVI (ml/m <sup>2</sup> )  |               |               |               |                |                                |                                      |
| Young                     | 44 $\pm$ 3    | 43 $\pm$ 3    | 39 $\pm$ 3    | $\leq 0.0001$  | NS                             | -13 $\pm$ 4                          |
| Older                     | 39 $\pm$ 2    | 35 $\pm$ 3    | 32 $\pm$ 2    |                |                                | -19 $\pm$ 3                          |
| CI (l/m <sup>2</sup> )    |               |               |               |                |                                |                                      |
| Young                     | 2.9 $\pm$ 0.2 | 4.1 $\pm$ 0.3 | 4.4 $\pm$ 0.4 | $\leq 0.0001$  | $\leq 0.0002$                  | 53 $\pm$ 9                           |
| Older                     | 2.4 $\pm$ 0.1 | 3.0 $\pm$ 0.2 | 3.0 $\pm$ 0.2 |                |                                | 22 $\pm$ 4*                          |

\*p < 0.01 for old vs. young on % change with atropine.

ANOVA = analysis of variance; BP = blood pressure; CI = cardiac index; EDVI = end-diastolic volume index; EF = ejection fraction; ESVI = end-systolic volume index; SVI = stroke volume index.

aging. Thus, the current study supports the conclusion that vagal activity has minor, if any, effects on ventricular contractility in resting humans who have low basal levels of sympathetic tone.

Differences in baseline vagal activity with age appear important in BP control. Older men had a greater decline than young men in mean BP (-40% vs. -20%) following ganglionic blockade (20). The greater reduction was due to a lesser increase in cardiac output, which in turn was due to a lower increase in heart rate (+3 vs. +25 beats/min), which

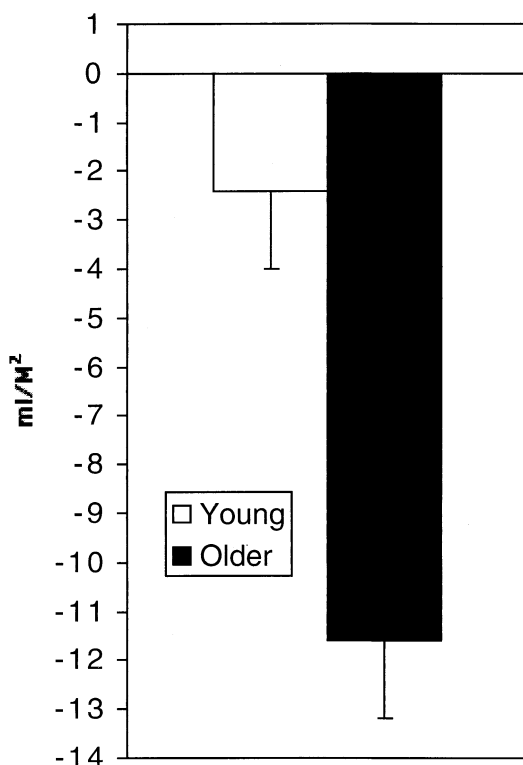
appeared due to a reduced basal tonic vagal control. Our findings of lesser changes in systolic and mean BP and heart rate and cardiac output with atropine in older subjects are concordant.

**DIASTOLIC FILLING.** The large increases in diastolic filling rates that occur during exercise depend on the ability of the ventricle to relax quickly and completely (31). Increased sympathetic activity increases diastolic filling (32,33), but there is less information regarding parasympathetic influ-

**Table 2.** Diastolic Filling Responses to Atropine by Age and Gender

|                                   | Rest          | 0.01 mg/kg    | 0.02 mg/kg    | ANOVA p Values |   |   |
|-----------------------------------|---------------|---------------|---------------|----------------|---|---|
|                                   |               |               |               | Dose<br>Effect | Young vs. Old $\times$<br>Atropine Dose | Female vs. Male $\times$<br>Atropine Dose |
| PEFR index in ml/s/m <sup>2</sup> |               |               |               | $\leq 0.0001$  | $\leq 0.04$                             | $\leq 0.04$                               |
| Young female                      | 255 $\pm$ 14  | 340 $\pm$ 17  | 390 $\pm$ 37  |                |   |   |
| Young male                        | 213 $\pm$ 28  | 268 $\pm$ 40  | 309 $\pm$ 49  |                |   |   |
| Older female                      | 163 $\pm$ 9   | 246 $\pm$ 28  | 251 $\pm$ 23  |                |   |   |
| Older male                        | 129 $\pm$ 21  | 140 $\pm$ 21  | 149 $\pm$ 28  |                |   |   |
| PEFR in EDV/s                     |               |               |               | $\leq 0.0001$  | $\leq 0.02$                             | $\leq 0.05$                               |
| Young female                      | 3.6 $\pm$ 0.2 | 5.1 $\pm$ 0.2 | 6.1 $\pm$ 0.4 |                |   |   |
| Young male                        | 3.3 $\pm$ 0.1 | 4.3 $\pm$ 0.3 | 5.6 $\pm$ 0.6 |                |   |   |
| Older female                      | 2.6 $\pm$ 0.1 | 4.2 $\pm$ 0.4 | 4.7 $\pm$ 0.3 |                |   |   |
| Older male                        | 2.0 $\pm$ 0.2 | 2.7 $\pm$ 0.2 | 2.9 $\pm$ 0.4 |                |   |   |

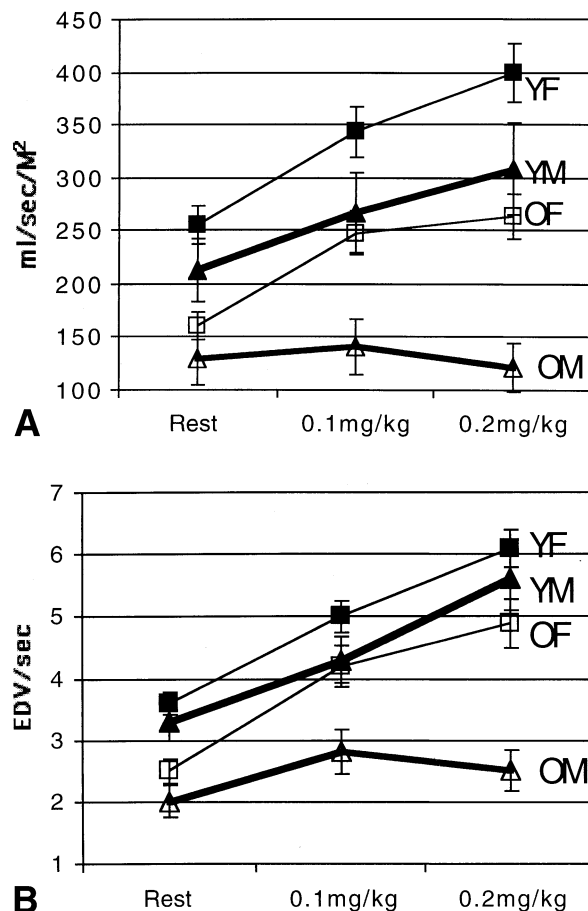
ANOVA = analysis of variance; EDV = end-diastolic volume; PEFR = peak early filling rate.



**Figure 2.** The decline in end-diastolic volume index following atropine at similar heart rates ( $94 \pm 2$  old vs.  $96 \pm 2$  young,  $p = \text{ns}$ ) and diastolic filling periods ( $340 \pm 13$  ms old vs.  $349 \pm 15$  ms young,  $p = \text{ns}$ ) was nearly fivefold greater in the old than in the young ( $-11.6 \pm 1.6$  ml/m<sup>2</sup> old vs.  $-2.4 \pm 1.6$  ml/m<sup>2</sup> young,  $p = 0.04$ ).

ence. In one study, both increases and decreases in vagal activity had no effect on tau, the time constant of left ventricular isovolumic pressure decay (29); however, a negative lusitropic effect of parasympathetic activity was demonstrable during concomitant increased sympathetic tone. In contrast, in animals there was no evidence of vagal influence on tau or chamber stiffness constant (6) in one study, but a clear decrease of tau with vagal stimulation in a different study (34); this same study (34) found that vagal stimulation in animals had a greater effect on tau than on contractile measures. Our findings are in accord in that we found no effect of vagal withdrawal on EF but a marked effect on diastolic filling. Our measure of diastolic filling is not analogous to tau, in that PEFR occurs somewhat later in early diastole than does tau, which occurs during isovolumic diastole.

The final phase of diastolic filling is due to atrial contraction, and atrial contraction is decreased by vagal activity (8,9). In one Doppler echocardiographic study, measures of HRV correlated inversely with A-wave velocity (35), raising the possibility that reduced parasympathetic tone with aging may account in part for the increase in A-wave velocity that occurs with aging. In another study (36), atropine caused lesser increases in Doppler echocardiography-measured A-wave peak in older subjects ( $p = 0.02$ ), consistent with a lower baseline vagal activity. In



**Figure 3.** Change in peak early diastolic filling in ml/s/m<sup>2</sup> (A) and in end-diastolic volume (EDV)/s (B) in young males (YM) and older males (OM) and young females (YF) and older females (OF) (both  $p < 0.01$  for young vs. old  $\times$  dose and  $p < 0.02$  for male vs. female  $\times$  dose).

the current study, parasympathetic withdrawal caused lesser increases in early diastolic filling rates in the older group. However, the PEFR that we measured occurs early in diastole and is unlikely to have been influenced by differences in atrial contraction.

Tachycardia induced by parasympathetic withdrawal shortens the cardiac cycle largely at the expense of diastole. In a prior study (37), similar atropine doses shortened the RR interval by 378 ms, of which 351 ms was due to shortening in diastolic filling time. The marked shortening of diastole with parasympathetic withdrawal is the probable cause of the drop in EDVI that occurred in both age groups. However, the older and probably stiffer ventricles were particularly vulnerable to shortening of diastole, having much greater declines in EDVI at a similar decrement in filling time. Thus, the older ventricle is less able to adapt to the demands of a tachycardia. The physiologic importance of the decline in parasympathetic withdrawal responses with aging is suggested by the correlation between the increase in heart rate following atropine and the peak heart rate achieved during maximal exercise testing in these same subjects ( $r = 0.79$ ,  $p \leq 0.0001$ ). Taken together, these

studies help establish the importance of changes in vagal tone on cardiovascular regulation with aging.

**Gender differences.** Few gender differences were seen. Resting PEFR in EDV/s was greater in the older females (all on hormone replacement) than in males (2.6 vs. 2.0 EDV/s), similar to a study by Spina et al. (2.4 vs. 2.0 EDV/s) in which hormone replacement status was not stated (38). We also found that older females had more preserved (i.e., greater) diastolic filling responses to parasympathetic withdrawal. The differences in filling rates in the older women and older men cannot be explained by factors known to directly or indirectly influence filling such as heart rate, EF, EDVI, or BP, which were similar in our study. In one prior study (39), estrogen replacement improved resting diastolic filling measured by echocardiography, and estrogen replacement improved endothelial function (40,41). Thus, it is possible that estrogen replacement influenced our results.

**Study limitations.** The observed hemodynamic changes represent both the primary effects of parasympathetic withdrawal and secondary reflex responses. The relative contributions cannot be determined. In addition, we cannot determine whether differences noted in diastolic filling with aging were simply consequent to tachycardia in a stiffer ventricle or were due to the elimination by atropine of a baseline parasympathetic lusitropic effect. This study is also unable to differentiate with certainty whether differences in responsiveness were due to a difference in vagal input to the heart or reduced tissue responsiveness. A weight-based atropine dose was given, and subjects received up to 2 mg, but we did not document complete blockade by giving an even higher dose.

The greater increase in systolic and mean BP in the young may have resulted in a greater decrease in the young in cardiac sympathetic activity due to the baroreflex. However, if this occurred, it would tend to lessen, not exaggerate, the observed age differences in heart rate and diastolic filling. In addition, in a prior study (37) we found no atropine effect on plasma catecholamines, thereby suggesting no atropine effect on sympathetic activity. Because all older females were receiving hormone replacement therapy, our results cannot be generalized to all older women.

**Significance of the study findings.** This study extends our understanding of the importance of changes in autonomic nervous system regulation of cardiac function with aging. The rigorous screening criteria make it unlikely that the observed differences are due to occult disease in the older group. Rather, the changes seen are more likely primary age-associated differences. This investigation demonstrates that the aging cardiovascular system is under lesser vagal control, and this is manifested by differences in heart rate, BP, and cardiac output responses to parasympathetic withdrawal. This in turn is likely primarily due to a reduction in baseline vagal tone. Additionally, diastolic filling responses decline substantially with age, particularly in men, with the older ventricle having lesser increases in diastolic filling rates

and greater declines in EDV with an increase in heart rate. These changes likely contribute to the greater prevalence of diastolic heart failure with aging, and they point to a potential mechanism underlying diastolic dysfunction in the elderly. Overall, these findings suggest a substantial decline in parasympathetic regulation of cardiac function with aging. Thus, cardiac responses to both sympathetic stimulation and to vagal withdrawal are attenuated with normal aging.

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